ARGUMENTS/REMARKS

The applicant and his counsel wish to thank the Examiner for discussing the Office Action mailed March 19, 2004 and merits of the application during a personal interview with the applicant and their counsel on April 20, 2004. The general nature of the interview has been summarized by the Examiner in the Interview Summary.

The applicant herein submits a formal response to the office action. In the office action, the Examiner withdrew the rejection of claim 12 under 35 U.S.C. 112, second paragraph, maintained the rejection of claims 1-12 and 16-27 under 35 U.S.C. 112, first paragraph, and raised new rejections under 35 U.S.C. 112, second paragraph and 35 U.S.C. 102. Each of the rejections is addressed separately below.

A 37 C.F.R. 1.132 declaration by Dr. Hideki Suzuki as well as 11 sets of "real world" use data are attached to demonstrate that the claims are enabled. The data shows that, as predicted in the application, the present invention can be used to kill bacteria on cut flowers, on meat and other human foods such as potatoes, in sheep blood plasma, and in human urine. The data was generated by and under the supervision of Dr. Hideki Suzuki, who has a Ph.D. in microbiology from the University of Tokyo and 16 years of post-doctoral experience in microbiology. Dr. Suzuki is currently employed as Director of Microbiology by ConjuGon, Inc. (Madison, WI), the licensee of the technology described in the present application.

In view of the claim amendments presented above, the arguments provided below, the declaration by Dr. Suzuki, and the data attached, reconsideration of the merits of the application is respectfully requested.

No extension of time is believed to be necessary and no fee is believed to be due in connection with this response. However, if any extension of time is required in this or any subsequent response, please consider this to be a petition for the appropriate extension and a request to charge the petition fee to the Deposit Account No. 17-0055. No other fee is believed to be due in connection with this response. However, if any fee is due in this or any subsequent response, please charge the fee to the same Deposit Account No. 17-0055.

I. NEW MATTER REJECTION UNDER 35 U.S.C. 112, FIRST PARAGRAPH (ITEM 4 OF OFFICE ACTION)

The Examiner maintained the new matter rejection against claims 1-12 and 16-27 stating that the claim amendment from "antibacterial agent" to "recombinant bacterium" is not supported by the specification. Although the applicant disagrees with the Examiner, in order to expedite prosecution, the applicant has further amended claims 1 and 16 to "a

recombinant donor bacterium ...," which is supported by the specification at page 14, line 21 and other related parts.

The amended claims are now drawn to a recombinant donor bacterium having a transmissible plasmid that comprises an origin of replication, an origin of transfer, and at least one screenable marker gene. In amended claims 1-12, the plasmid is a runaway plasmid and the initiation of replication in the recombinant bacterium is negatively controlled by a plasmid replication repressor. In claims 16-27, the plasmid further comprises a "killer gene".

At page 14, line 21, the term "recombinant donor bacteria" is specifically disclosed. The specification further discloses that the recombinant donor bacteria comprise the "killer plasmid". In one aspect, the specification defines the "killer plasmid" as a runaway plasmid (as in Claim 1) that contains an origin of replication wherein the initiation of replication in the recombinant bacteria is negatively controlled by a plasmid replication repressor, an origin of transfer, and at least one screenable marker gene (lines 14-15 of page 14, line 25 of page 3 to line 1 of page 4, lines 19 and 20 of page 5, lines 7-15, of page 8, and line 29 of page 8 to line 5 of page 9). In another aspect, the specification defines the "killer plasmid" as a plasmid (as in Claim 16) that contains an origin of replication, an origin of transfer, a "killer gene", and at least one screenable marker gene (lines 14-15 of page 14, lines 8-12 of page 4, lines 10-11 of page 13 (for screenable marker), and lines 15-17 of page 11). Therefore, the amended claims are supported by the specification.

In raising the rejection, the Examiner further stated that nowhere in the specification the term "recombinant bacterium" is defined as "antibacterial agents." The applicant respectfully submits that antibacterial properties are not recited in the claims and it is not appropriate for the Examiner to read such properties into the claims and then require support for the properties in the specification. Nevertheless, if for the sake of argument this limitation is imported, the term *is* supported by the specification. As described above, the recombinant donor bacteria of the present invention comprise "killer plasmids" that, upon transfer to a recipient bacterium, kill said recipient bacterium (see, *e.g.*, lines 15-22 of page 5 and lines 15-20 of page 11). This strategy is specifically described as an "antibacterial" strategy (*e.g.*, line 15 of page 5).

II. LACK OF ENABLEMENT REJECTION UNDER 35 U.S.C. 112, FIRST PARAGRAPH (ITEM 5 OF OFFICE ACTION)

The Examiner maintained the lack of enablement rejection based on Ambrozic et al., Klimke et al., Roberts et al., and Rahal et al. (i). The Examiner also alleged that: (ii) the applicant used a pathogenic microorganism *E. coli* as the donor strain; (iii) the applicant asserted that it is well known in the art to use the claimed invention to eliminate bacteria from animal feed; and (iv) the Filutowicz declaration does not support that the invention was enabled at the time of filing.

The applicant respectfully disagrees on all points. Under subsection A below, the applicant will explain how Dr. Suzuki's declaration and the attached data demonstrate that the specification enabled real world uses specified in the application. Under subsection B, the applicant will address each of the points raised by the Examiner.

A. Real world use is enabled: Dr. Suzuki's declaration and the data attached.

The applicant first notes an invention can be enabled even though some level of experimentation is necessary. In re Angstadt, 537 F. 2d 498 (CCPA, 1976). In determining whether the claims are enabled, the key inquiry is not whether any experimentation is necessary, but rather whether the experimentation is undue. In re Angstadt, 537 F. 2d 498 (CCPA, 1976). If the experimentation is merely routine, a considerable amount of experimentation is permissible. In re Wands, 858 F. 2d 731 (Fed. Cir. 1988).

The applicant further notes that Dr. Suzuki's declaration and the attached data are submitted to demonstrate that a skilled artisan can practice the invention by following the teachings of the specification. The declaration and attached data are not submitted to supplement the specification with the experimental details. Any experimental details in the attached data that are not in the specification are not necessary for enablement because they only involve techniques that were known and routine at the time the application was filed. Specific references for relevant routine techniques known at the time of filing are provided with the attached declaration and data.

At the interview of April 20, 2004, the Examiner requested that the applicant provide experimental data showing "real world" uses of the claimed invention on biological sample materials. The 11 sets of experimental data attached demonstrate that a skilled artisan can readily follow the teaching of the present invention to kill bacteria on biological samples such as cut flowers as described at page 16, line 21 of the application (see the flower petal and leaf data attached), on meat and other foods as described at page 16, lines 17-18 of the application

(see the meat and sliced potato data attached), and in biological fluids such as would be encountered in intravenous or urogenital applications, as provided at page 14, line 26 of the application (see the blood plasma and urine data attached).

As Dr. Suzuki stated in the declaration, the attached data was generated by following the teachings of the specification using technologies that were known and routine at the time the application was filed. The data demonstrates the killing of recipient bacteria by conjugative transfer of a transmissible plasmid from a donor bacterium. The donor and recipient cells used in the experiments are those described in the specification. The transmissible plasmids were also constructed based on the teachings of the specification. The exact parts of the specification that teach the donor and recipient bacteria as well as the plasmids that were employed in the experimentation are detailed below, and a summary is provided in Table 1 (copied from the declaration attached). The applicant notes that only one use needs to be enabled for the pending composition claims (see MPEP 2164.01(c), 4th paragraph). Nonehteless the applicant submits data on multiple uses to emphasize that a skilled artisan can readily practice the present invention by following the teachings of the specification.

For the experimentation attached, killing was through the use of "killer genes" as described at page 11, line 15 to page 13, line 6, and also as described in claim 16. A non-self-transmissible plasmid, pCON4-44, and a self-transmissible plasmid, pCON4-47, were used for the experiments. The genetic elements of these plasmids are shown in Fig. 2 attached to Dr. Suzuki_'s declaration. As taught on page 9 at lines 12-19 of the application, non-self transmissible plasmids generally comprise an *oriT*, an *oriV* and one or more screenable markers. The non-self transmissible plasmid used in these tests, pCON4-44, comprises *oriT* and *oriV* and includes the *TetR* tetracycline resistance gene as a screenable marker. As taught on page 9 at lines 20-27, self-transmissible plasmids generally comprise these same elements, and additionally comprise *tra* transfer genes. The self-transmissible plasmid used in these tests, pCON4-47, comprises *oriT*, *oriV*, and the TetR screenable marker and further comprises Tral and Tra2 regions encoding the whole set of the *tra* transfer genes.

colE3 was used as a "killer gene". Colicin E3 is a ribonuclease that cleaves 16S ribosomal RNA, inhibiting synthesis of proteins in the cell and leading to cell death, which were well known in the art at the time the application was filed (Diaz et al. Mol. Microbiol 13:855-861, 1994). This *colE3* gene was integrated in the two above plasmids (pCON4-44 and pCON4-47) using standard cloning techniques, in accordance with the methods of Sambrook and Ausubel, as cited at page 16, lines 23-27 of the present application. A self-

transmissible control plasmid, pCON4-45, was also constructed. pCON4-45 does not carry the "killer gene" but confers tetracycline resistance via conjugation, allowing conjugation efficiencies to be monitored without killing the recipient cell.

Three donor strains, each carrying one of the plasmids described above, were tested for conjugative transfer and recipient cell killing ability in and on a variety of biological samples and tissues including flower petals, leaves, meat, sliced potatoes, plasma, and urine. The donor strains were genetically engineered to contain abundant repressor, LacI, to repress the expression of the "killer gene" (*colE3*) according to the teaching of the specification (page 4, lines 14 and 14, page 13, lines 7-9, and claims 16 and 19). Expression of *colE3* on the plasmids was controlled by the *lac* promoter, *lacP*, which was negatively regulated by the abundant repressor I. Abundance of the repressor was well known in the art at the time of filing (Muller-Hill et al. Proc Natl Acad Sci 59:1259-1264, 1968; Amann et al. Gene 69:301-315, 1988; Kleiner et al. J Gen Microbiol 134:1779-84, 1988). The donor cells were also modified to contain the toxin-immunity protein ImmE3 (which can bind to ColE3 to prevent its toxic effect) so that any leaky expression of *colE3* would not cause any detrimental effect to the donor cells. Use of ImmE3 to prevent toxic effects of ColE3 was well known in the art when the application was filed (Bowman et al. Proc Natl Acad Sci 68:964-968, 1971; Masaki and Ohta. J Mol Biol 182:217-227, 1985).

In these experiments, two pathogenic bacteria, *Escherichia. coli* O157:H7 and *Salmonella typhimurium* (also referred to as *Salmonella enterica* serotype Typhimurium), were used as targets. These target pathogens are disclosed at page 15, lines 25-26.

In these experiments, equal amounts of donor cells carrying one of the "killer plasmids" (pCON4-44 or pCON4-47) and the pathogenic bacterial cells were mixed and spun down to a pellet. The pellet of mixed bacteria was re-suspended in plasma or urine. For testing on the surfaces of flower petals, leaves, meat, and sliced potatoes, the mixed bacterial pellet was re-suspended in a small volume of saline and spread on the surfaces. After one hour of incubated at 37°C, the mixture of bacteria was serially diluted, and survival of the target bacteria was monitored by growing them on nutrient-rich agar plates containing appropriate antibiotics. Unlike the filter conjugation described by Merryweather, which was standard and well known in the art at the time the application was filed (J. Bacteriol. Merryweather et al., 167:12-17, 1986; J. Bacteriol. Haase et al. 177:4779-4791, 1995), these experiments were carried out within or on the surface of biological materials. An illustration

of the method to monitor conjugation-dependent killing is shown in Fig. 1 attached to Dr. Suzuki's declaration.

The results of conjugation-dependent killing are shown in the 11 data sets attached to Dr. Suzuki's declaration. The results show that the "killer plasmids" (pCON4-44 and pCON4-47) could kill either 100% or very close to 100% of the exconjugants obtained from the two pathogenic bacteria under the conditions used. More details can be found in the 11 sets of data attached.

The data on flower petals, leaves, meat surfaces, and sliced potatoes demonstrates that a skilled artisan can readily use the present invention to kill bacteria on cut flowers and meat and other human foods as predicted in the specification (at page 16, lines 17-18 and 21). The plasma and urine data demonstrates that a skilled artisan can readily use the present invention to kill bacteria in human tissues and fluids by following the teachings of the present invention. Pharmaceutical preparations involving bacteria in general are known in the art, and were known at the time of filing of the present application (see, *e.g.*, US Patent Nos. 5,032,399 issued July 1991 to Gorbach et al., 5,733,568 issued March 1998 to Ford, and 4,314,995 issued February 1982 to Hata et al. for other bacterial pharmaceuticals). For a particular pharmaceutical application, a skilled artisan can readily determine the suitable dosage and formulations through routine experimentation such as is frequently conducted in the art. Since only one use needs to be enabled for the composition claims (MPEP 2164.01(c), 4th paragraph), any one set of data discussed above is believed to be sufficient to show that the invention is enabled by the specification.

Table 1.

Claim Element (by reference to Claim 16)	**Component used in present tests**	**Citation examples in specification**
Bacterial cell harboring the whole set of the *tra* transfer genes on its chromosome	*E. coli* strain S17-1	*E. coli* as donor cell: page 13, lines 25-17.
Transmissible plasmid comprising	pCON4-47, pCON4-44	Embodied in the Description of the Invention, as a whole,
An origin of replication	RK2 *oriV*	*oriV* teachings at Page 10, lines 8-21, including RK2 specifically
An origin of transfer	RK2 *oriT*	*oriT* teachings at Page 9, lines 2-3; attached Fig. 2
A screenable marker gene	TetR, AmpR, KanR,(antibiotic resistance markers);	Page 13, line 15 (general teaching of antibiotic resistance genes as suitable screenable markers)
One or more transfer genes conferring the on the donor bacterium the ability to conjugatively transfer the transmissible plasmid to the recipient bacterium	Tra1 and Tra2 on plasmid pCON4-47	Sources for transfer (*tra*) genes: Page 11, lines 3-14
"Killer gene"	*colE3*	"Killer genes", generally at page 11, line 15- page 13, line 6; *colE3*, known in the art at the time of filing: (Bowman et al. Proc Natl Acad Sci 68:964-968, 1971)
Pathogenic recipient bacterium	*E. coli* 0157:H7	*E. coli* linked to enteritis, page 15, lines 25-26
Pathogenic recipient bacterium	*Salmonella typhimurium*	*Salmonella typhimurium* at page 15, line 26

B. Points raised by the Examiner.

(i) The references cited do not show that there is uncertainty regarding plasmid stability.

Plasmid manipulation, including, construction of plasmids having particular genetic elements (*e.g.*, selectable markers for selecting and/or identifying stable transformants), transformation of bacteria to contain such plasmids, testing to determine success or transformation, and growth and storage of bacteria harboring such plasmids is old in the art.

See, *e.g.*, Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory (1989) or Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (2000), cited in the present application at page 16, lines 25-27. Sambrook teaches features that should be included on a plasmid to confer the desired functionality, and further teaches the use of selectable markers to stably establish plasmids in transformed cells. In view of the body of work known in the art, the four references cited by the Examiner do not suggest that manipulation and maintenance of plasmids is an unpredictable art.

These references identify and characterize factors that have been found to be related to plasmid function in particular hosts, or during transfer between particular hosts. Rather than showing that there is uncertainty regarding the use of plasmids, these references demonstrate that, in the event that a plasmid behaves in an unusual or defective fashion, those of skill in the art are well able to detect and characterize behaviors using standard techniques.

In addition, the claims specify that the transmissible plasmid comprises a selectable marker. The use of selectable markers in stabilizing plasmids in host cells is well known in the art. See, *e.g.*, Sambrook et al., *supra.* The references cited do not suggest that there is uncertainty regarding the use of plasmids having selectable markers in the creation of donor host cells comprising such plasmids.

As Dr. Filutowicz explained in his declaration filed on November 19, 2003, transferring plasmids from donor cells to recipient cells can be readily accomplished by a skilled artisan (paragraph 7 of the declaration). In fact, it is routinely conducted in the art (id.). Stability is usually not a problem. The data submitted along with Dr. Filutowicz's declaration as well as the data submitted with this response are consistent with this notion.

Furthermore, an invention can be enabled even if certain embodiments are not operative. Even assuming for the sake of argument that certain specific embodiments may be inoperable due to plasmid instability, inclusion of any such embodiments within the scope of the claims does not necessarily render the claims nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. See MPEP 2164.08(b), citing Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) (prophetic examples do not make the disclosure nonenabling). As noted above, methods related to plasmid manipulation and testing are old in the art. As Dr. Filutowicz's declaration of November 19, 2003 and Dr. Suzuki's declaration submitted herewith, a skilled artisan is familiar with transferring a plasmid from a donor cell to a recipient cell and thus would be

able to make these determinations (whether an embodiment is operative or inoperative). The additional information regarding factors related to stability described in the four references cited by the Examiner can only help a skilled artisan to make such determinations.

(ii) E. coli is not always a pathogenic organism

The Examiner asserts that "It is well known that *E. coli* is a pathogenic microorganism." Office Action at page 6. The applicant respectfully disagrees with this characterization. While it is true that some strains of *E. coli* are pathogenic, non-pathogenic strains of *E. coli* are well known and are widely used in the art. See, *e.g.*, Sambrook, *et al.*, *supra*.

The present application discloses specific examples of non pathogenic strains of *E. coli*. See, *e.g.*, strain F18 and Nissle 1917 (page 13, lines 26 and 27 of application). In addition, criteria for selecting non-pathogenic strains of *E. coli* are well known to those skilled in the art. For example, the NIH Guidelines for Research Involving Recombinant DNA Molecules classifies microbes according to risk group, and states that a strain of *Escherichia coli* is an RG1 agent (RG1 = "Agents that are not associated with disease in healthy adult humans") if it (1) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen); and (2) does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors. http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html, Appendix B. Thus, skilled artisans are readily able to identify strains of *E. coli* that are suitable for use in the presently claimed invention.

(iii) Applicant did not assert that it is well known in the art to use the claimed invention to eliminate bacteria from animal feed.

The Examiner has alleged that the applicant asserted "that it is well known in the art to use the claimed invention to eliminate bacteria from animal feed." Office Action at page 6. This does not accurately represent the applicant's assertions. Rather, the applicant stated that, following the teaching of specification, a skilled artisan can eliminate bacteria from animal feed without undue experimentation (see page 5, lines 1-3 and 9-13 of the response to last office action and lines 4-7 of paragraph 3 of the Filutowicz Declaration). Furthermore, the conditions employed to generate the data submitted with the Filutowicz declaration are suitable to eliminate pathogenic bacteria in animal feed.

Bacterial animal feed additives were known in the art at the time of filing (see, *e.g.*, US Patent No. 5,549,890, issued August 27, 1996 to Kubo, describing the use of *Bacillis subtilis* in livestock feed to produce a fattening feed; US Patent No. 4,138,498, issued February 6, 1979 to Das, *et al.*, describing the use of cultures of *Megasphaera elsdenii* and *Selenomonas ruminantium* as feed additives for ruminant animals to improve dietary adaptation). Thus, methods for treating animal feed with useful bacteria were known in the art at the time of filing. In addition, the use of antibiotic feed additives to kill bacteria is well known in the art, as it was at the time of filing (see, *e.g.*, US Patent No. 5,549,890, issued August 27, 1996 to Kubo; US Patent No. 4,138,498, issued February 6, 1979 to Das, *et al.* Use of antibiotic and antibacterial compositions in animal feed: see, *e.g.*, US Patent No. 5,266,347 issued November 30, 1993, to King). The present application discloses using bacteria having antibacterial properties as treatment for animal feed. The present application, when read in view of that known in the art at the time of filing, enables the recited uses in animal feed.

(iv) Data submitted with the Filutowicz declaration supported that the invention was enabled at the time the application was filed.

As Dr. Filutowicz explained in his declaration, the data submitted with the declaration was generated by simply following the teaching of the specification. In other words, the researchers read the specification and did the experiments. The details of the experiments provided in the declaration are not necessary for enablement because the experiments submitted with the declaration were conducted without these details. The Filutowicz declaration was filed not to provide the experimental details but to demonstrate that a skilled artisan can practice the invention by following the teachings of the specification. Therefore, the claims were enabled at the time the application was filed.

III. CLARITY REJECTION DIRECTED TOWARDS "NON-PATHOGENIC CONJUGATIVE DONOR BACTERIUM" (ITEM 6 OF OFFICE ACTION)

The Examiner rejected claims 1-12 under 35 U.S.C. 112, second paragraph stating that the term "non-pathogenic conjugative donor bacterium" recited in claim 1 is unclear. The term has been deleted from claim 1 and the rejection is moot.

IV. ANTICIPATION REJECTIONS (ITEMS 7 AND 8 OF OFFICE ACTION)

A. Anticipation rejection based on Metcalf et al. (item 7 of office action).

The Examiner rejected claims 1, 3-5, and 7-12 under 35 U.S.C. 102(b) stating that the claims are anticipated by Metcalf et al. (Plasmid, 1996, 35: 1-13). The applicant respectfully disagrees. Claim 1 and dependent claims recite as a claim element that the transmissible plasmid is capable of undergoing runaway replication. This element is not disclosed by Metcalf et al. The applicant further notes that this rejection had been raised once before in the office action mailed November 27, 2001 and withdrawn by the Examiner in the subsequent office action mailed July 12, 2002.

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. MPEP 2131, citing Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d. 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Metcalf et al. does not teach a plasmid capable of undergoing runaway replication in a recipient bacterium.

As explained in more detail below, the plasmids in Metcalf et al. cannot undergo runaway replication. Moreover, the plasmids in Metcalf et al. are in fact designed not to replicate in recipient cells at all to facilitate the integration of certain parts of the plasmids into the genome of the recipient cells. In contrast, the plasmid recited in the claims is designed to undergo runaway replication in the recipient cells to kill the recipient cells.

As stated in claim 1, the transmissible plasmid recited therein undergoes runaway replication in the absence of a plasmid replication repressor. In other words, the replication of the plasmid is negatively controlled by the repressor. In the donor cells wherein the repressor is present, the replication of the plasmid is inhibited. In the recipient cells wherein the repressor is absent, the plasmid undergoes runaway replication to kill the recipient cells.

The plasmids disclosed in Metcalf et al., however, cannot undergo runaway replication because their replication is positively controlled by the π protein. See lines 1-5 of the abstract of Metcalf et al. In other words, the plasmids replicate in the presence of π protein and do not replicate in the absence of π protein. Even in the presence of the π protein, the plasmids only undergo regulated replication but not runaway replication. Even though they can exist in high copy numbers, high copy numbers still reflect regulated and controlled replication but not runaway replication.

Furthermore, in Metcalf et al., plasmid replication in the recipient cells is suppressed due to the absence of the π protein. See lines 12-15 of the abstract of Metcalf et al. The plasmids in Metcalf et al. are the so-called suicide plasmids designed for integrating into the

genome of the recipient cells. Lack of replication in the recipient cells is essential for the selection of the integration events. Thus, Metcalf et al. does not teach a plasmid capable of undergoing runaway replication in a recipient bacterium. As such Metcalf et al. fails to teach each and every element of Claims 1, 3-5, and 7-12, and thus does not anticipate these claims. For these reasons, the applicant respectfully requests that this rejection be removed.

B. Anticipation rejection based on Kaniga et al. (item 8 of office action).

The Examiner rejected claims 1, 3-5, 7, 9, 11, 16, 18, 19, 24, and 16 under 35 U.S.C. 102(b) stating that the claims are anticipated by Kaniga et al. (Gene, 1991, 109: 137-141). The applicant respectfully disagrees.

With regard to claims 1, 3-5, 7, 9, and 11, Kaniga et al. cannot anticipate these claims because the reference does not disclose the claim element that the plasmid is capable of undergoing runaway replication in recipient cells.

The same arguments provided above for Metcalf et al. apply here. As in Metcalf et al., the plasmids disclosed in Kaniga et al. (pKNG101 and pKNG105) cannot undergo runaway replication as their replication is positively controlled by the π protein. See Kaniga et al., the last three lines of the left column on page 138, the legend of Fig. 1 on page 138, and the last paragraph of the right column on page 139, which indicate that the origin of replication on pKNG101 and pKNG105 is the oriR6K described in Miller and Mekalano, 1998, which states the replication from oriR6K requires the product of the pir gene, the π protein at the left column, lines 9 and 10 under the subsection of "construction of pJM703.1" on page 2576 (a copy of Miller and Mekalano, 1998 is attached). Further as in Metcalf et al., the plasmids disclosed in Kaniga et al. are suicide plasmids that are designed not to replicate in the recipient cells to facilitate their integration into the genome of the recipient cells. See the left column, lines 5-10 on page 140 of Kaniga et al. Therefore, Kaniga et al. does not anticipate claims 1, 3-5, 7, 9, and 11.

With regard to claims 16, 18, 19, 24, and 26, Kaniga et al. cannot anticipate these claims because the reference does not disclose the claim element that the donor cell is modified so as to be unaffected by the product of the "killer gene".

The claims at issue require that the donor cells be modified so as not to be affected by the "killer gene" whereas the recipient cells are not so modified and thus sensitive to the "killer gene". In Kaniga et al., *sacB* is the alleged "killer gene". The *sacB* product is lethal to bacteria only in the presence of sucrose. Kaniga et al. does not teach modifying the donor cells so that they will not be killed upon exposure to sucrose. There is no need to modify the

donor cells in Kaniga et al. because the donor cells are not exposed to sucrose. The plasmids of Kaniga et al. are suicide plasmids that are designed for integration into the genome of the recipient cells and *sacB* was genetically engineered into the plasmids as a selection tool for the plasmid excision event needed for the integration. The plasmids are transferred from the donor cells to the recipient cells and the recipient cells are exposed to sucrose to select for the excision event. Therefore, both the donor cells and the recipient cells in Kaniga et al. are sensitive to the "killer gene" whereas only the recipient cells are sensitive to the "killer gene" in the claims at issue. Accordingly, Kaniga et al. cannot anticipate the claims. For these reasons, the applicant respectfully requests that this rejection be removed.

V. CLARITY REJECTION DIRECTED TOWARDS "DERIVED FROM" AND "OBTAINED FROM" (ITEMS 9-11 OF OFFICE ACTION)

The Examiner rejected claims 1-12 under 35 U.S.C. 112, second paragraph stating that the term "derived from" recited in claims 9 and 12 is not clear. In response, the applicant amended claims 9 and 12. Instead of stating that the origin of replication or transfer genes are "derived from" certain plasmids, amended claims 9 and 12 now state that they <u>are</u> origins of replication or transfer genes of the these plasmids. This rejection is believed to have been overcome by the amendment.

The Examiner rejected claims 16-27 under 35 U.S.C. 112, second paragraph stating that the term "derived from" recited in claims 24 and 27 is not clear. In response, the applicant amended claims 24 and 27. Instead of stating that the origin of replication or transfer genes are "derived from" certain plasmids, amended claims 24 and 27 now state that they <u>are</u> origins of replication or transfer genes of the these plasmids. This rejection is believed to have been overcome by the amendment.

The Examiner rejected claims 16-27 under 35 U.S.C. 112, second paragraph stating that the term "obtained from" recited in claim 20 is not clear. In response, the applicant amended claim 20. Instead of stating that a "killer gene" is "obtained from" a bacteriophage, amended claim 20 now states that the "killer gene" <u>is</u> a "killer gene" of a bacteriophage. This rejection is believed to have been overcome by the amendment.

VI. WITHDRAWN OF REJECTION OF CLAIM 12 UNDER 35 U.S.C. 112, SECOND PARAGRAPH (ITEM 3 OF OFFICE ACTION)

At item 3 of the office action, the Examiner stated that the rejection of claim 12 under 35 U.S.C. 112, second paragraph is withdrawn. The applicant could not find a rejection of claim 12 under 35 U.S.C. 112, second paragraph in the previous office action mailed July 29, 2003. Clarification is respectfully requested.

Respectfully submitted,
Marcin S. Filutowicz

June 15, 2004

By: _____

Zhibin Ren
Reg. No. 47,897
Attorneys for Applicants
Quarles & Brady LLP
411 East Wisconsin Avenue
Milwaukee, WI 53202-4497

TEL (414) 277-5633
FAX (414) 271-3552

QBMKE\5584933.1